

ABSTRACT

The present invention provides non-radioactively labeled synthetic substrates for enzymatic reactions which exhibit markedly improved solubility having the general

5 structure  $*F-R_1-L_1-R_2-P_{Hc1}-P_S-P_{Hc2}-(R_3-L-R_4-T)_y$ . These substrates may be designed to carry a charge to allow electrophoretic separation of substrates and reaction products. The invention also provides enzymatic activity assays for protein kinases, phosphatases and proteases utilizing the substrates of the invention, as well as methods of producing these

10 substrates. In addition, the invention also provides libraries of the substrates, and methods of utilizing these libraries to select optimal synthetic peptide enzyme substrates for high-throughput screening assays.